

WHAT IS CLAIMED:

- Sub B4
1. A method of rejuvenating a primary cell, comprising:
    - a. transferring a primary cell, the nucleus from said primary cell or chromosomes from a primary cell to a recipient oocyte or egg in order to generate an embryo;
    - b. obtaining an inner cell mass, embryonic disc and/or stem cell using said embryo;
    - c. injecting said inner cell mass, embryonic disc and/or stem cell into an immune-compromised animal to form a teratoma;
    - d. isolating said resulting teratoma;
    - e. separating the different germ layers for the purpose of identifying specific cell types;
    - f. isolating a cell of the same type as the primary cell.
  - 15 2. The method of Claim 1, wherein said primary cell is a senescent cell or a cell that is near senescence.
  - 20 3. The method of Claim 1, wherein said cell isolated from said nuclear transfer teratoma has telomeres that are on average at least as long as those of cells from a same age control teratoma that is not generated by nuclear transfer techniques.

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4. The method of Claim 4, wherein said telomeres are on average longer

than those of cells from a same age control teratoma that is not generated by nuclear transfer techniques.

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5. The method of Claim 2, wherein said primary cell is a fibroblast.

6. The method of Claim 1, wherein said immune-compromised animal is a SCID or nude mouse.

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7. The method of Claim 1, wherein said primary cell has at least one alteration to the genome.

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8. A method of making a primary cell having the same genotype as a first cell which is of a different cell type, comprising:

- a. transferring the nucleus from said first cell to a recipient oocyte in order to generate an embryo;
- b. obtaining an inner cell mass, embryonic disc and/or stem cell using said embryo;
- c. injecting said inner cell mass, embryonic disc and/or stem cell into an immune compromised animal to form a teratoma;
- d. isolating said resulting teratoma;

- e. separating the different germ layers for the purpose of identifying specific cell types;
- f. isolating a cell of a different type than the first cell,

wherein the telomeres of said new primary cell are at least as long the telomeres of a same age control cell in a teratoma not generated by nuclear transfer techniques.

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- 9. The method of Claim 8, wherein said first cell is a senescent cell or a cell that is near senescence.

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- 10. The method of Claim 9, wherein said first cell is a fibroblast.

- 11. The method of Claim 8, wherein said primary cell is of a type selected from the group consisting of smooth muscle, skeletal muscle, cardiac muscle, skin and kidney.

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- 12. The method of Claim 8, further comprising growing said cell of a different type in the presence of growth factors to facilitate further differentiation.

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- Sub B7
- 13. The method of Claim 11, wherein said primary cell is used to generate a tissue (for transplantation into a patient in need of a transplant).

14. The method of Claim 8, wherein the genome of the first cell is altered prior to nuclear transfer.

15. The cell isolated by the method of Claim 8.

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16. The tissue isolated by the method of Claim 13.

17. The method of Claim 7, wherein said genetic alteration comprises the transfection of at least one heterologous gene.

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18. The method of Claim 7, wherein said genetic alteration comprises the disruption of at least one native gene.

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19. The method of Claim 14, wherein said genetic alteration comprises the transfection of at least one heterologous gene.

20. The method of Claim 14, wherein said genetic alteration comprises the disruption of at least one native gene.

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*sub B8* 21. A method of performing compound genetic manipulations in a primary cell, comprising rejuvenating said primary cell between genetic manipulations using

nuclear transfer into a recipient oocyte, wherein said cell is passaged to a senescent or near-senescent state prior to nuclear transfer.

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like or near-senescent-like state prior to nuclear transfer.

10           23. The method of Claim 21, whereby rejuvenation results in an embryonic cell that has telomeres at least as long on average as a same age control embryonic cell.

15           24. A primary cell that has been genetically altered according to the method of Claim 21.

20           Sub B<sup>9</sup> 25. A method of making a genetically altered animal having the same genotype as the cell of Claim 24, comprising

- a. transferring the nucleus of said cell into a recipient oocyte,
- b. generating an embryo or embryonic stem cell from said nucleated oocyte,

c. introducing said embryo or embryonic stem cell into a recipient female,

and

d. allowing said embryo or embryonic stem cell to fully develop such that  
said female delivers a newborn animal having the same genotype as said  
primary cell.

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26. The genetically altered animal produced by the method of Claim 25,

whereby said animal has telomeres that are at least as long on average as a same age  
control animal.

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27. A method of re-cloning a cloned animal using nuclear transfer

techniques, wherein the donor cell used to supply the nucleus of the re-clone is a cell  
that is senescent or near senescence.

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28. The method of Claim 25, wherein said re-cloned animal has been

genetically altered with respect to the cloned animal.

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29. A method of making a re-cloned inner cell mass, blastocyst, teratoma  
embryo, fetus or animal containing at least two genetic modifications, comprising:

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a. obtaining a primary cell from an animal of interest,

- b. making a first genetic modification to said primary cell by inserting heterologous DNA and/or deleting native DNA,
- c. allowing said genetically modified primary cell to multiply to senescence or near-senescence,
- 5 d. using a first genetically modified senescent or near-senescent cell as a nuclear donor for nuclear transfer to an enucleated oocyte or an enucleated fertilized egg,
- e. obtaining a cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal having said first genetic modification,
- 10 f. obtaining a cloned primary cell from said cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal,
- g. making a second genetic modification to said cloned primary cell by inserting heterologous DNA and/or deleting native DNA,
- 15 h. allowing said second cloned primary cell to multiply until senescence or near senescence,
- i. using a senescent or near-senescent cloned primary cell having said first and second genetic modifications as a nuclear donor for nuclear transfer to an enucleated oocyte or an enucleated fertilized egg, and
- 20 j. obtaining a re-cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal having said first and second genetic modifications.

30. The method of Claim 29 further comprising steps where said re-cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal is again re-cloned, and wherein a third genetic modification is made such that the further re-clone has the first, second and third genetic modifications.

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31. The method of Claim 30, wherein said further re-clone is generated by nuclear transfer techniques using a senescent or near-senescent donor cell.

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32. The method of Claim 29, wherein said re-clone has telomeres that are at least as long on average as a same age control animal that was not generated using nuclear transfer techniques.

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33. The method of Claim 31, wherein said further re-clone has telomeres that are at least as long on average as a same age control animal that was not generated using nuclear transfer techniques.

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34. The method of Claim 29, wherein the genetic modifications involve genes that are responsible for immunological function.

35. The method of Claim 29, wherein said animal of interest is an ungulate.

36. The method of Claim 35, wherein said animal of interest is a bovine.

Sub B<sup>17</sup> 37. A method of re-setting the lifespan of senescent or near-senescent cells,  
comprising transferring the nucleus of said cell into a recipient oocyte.

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38. The method of Claim 37 wherein said recipient oocyte is of a different  
species than said senescent or near-senescent cell.

10 39. The method of Claim 37 further comprising generating an embryo or

embryonic stem cell from said nucleated oocyte.

15 40. A method of identifying at least one gene that either directly or indirectly  
enhances telomerase activity, comprising, screening a cDNA or mRNA library  
generated from an embryo or embryonic stem cell for members that enhance  
telomerase activity in a senescent or near-senescent cell.

20 41. The method of Claim 40 whereby enhancement in telomerase activity is  
measured by measuring for enhanced expression of a telomerase reporter gene.

42. The method of Claim 41 wherein said telomerase reporter gene is a  
construct comprising the hTRT gene fused to a reporter gene.

43. The method of Claim 42 wherein the construct comprises a gene fusion.
44. The method of Claim 42 wherein the construct comprises a protein fusion.
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45. The method of Claim 40 whereby enhanced telomerase activity is measured via the TRAPeze assay.
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46. The method of Claim 40 whereby said cDNA or mRNA library is subjected to subtractive hybridization with a cDNA or mRNA library from a senescent cell prior to library screening.
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47. A method of identifying at least one gene that either directly or indirectly suppresses telomerase activity, comprising, screening a cDNA or mRNA library generated from a senescent or near-senescent cell for members that suppress telomerase activity in an embryonic stem cell .
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48. The method of Claim 47 whereby a decrease in telomerase activity is measured by measuring for decreased expression of a telomerase reporter gene.

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49. The method of Claim 47 wherein said telomerase reporter gene is a construct comprising the hTRT gene fused to a reporter gene.
50. The method of Claim 49 wherein the construct comprises a gene fusion.
51. The method of Claim 49 wherein the construct comprises a protein fusion.
52. The method of Claim 47 whereby telomerase activity is decreased via a protein interaction, and a decrease in telomerase activity is measured via the TRAPeze assay.
53. The method of Claim 47 whereby said cDNA or mRNA library is subjected to subtractive hybridization with a cDNA or mRNA library from an embryonic stem cell prior to library screening.

54. A method of identifying a protein that enhances telomerase activity, comprising
- collecting fractions from the cytoplasm of an oocyte,
  - adding them to a cell-free system designed from a senescent or near-senescent cell, and

c. measuring for changes in telomerase activity that result from exposure to specific oocyte cytoplasmic fractions.

5 55. A gene identified by the method of Claim 40.

56. A gene identified by the method of Claim 47.

10 57. A protein identified by the method of Claim 54.

15 58. A method for screening for compounds that inhibit telomerase activity, comprising exposing an embryonic stem cell generated by nuclear transfer techniques using a senescent or near-senescent donor cell to a compound to determine whether said compound inhibits telomerase activity.

15 59. A compound identified by the method of Claim 58.

20 60. A pharmaceutical composition comprising the gene of Claim 55, or a portion or a transcription product thereof, for the purpose of enhancing telomerase activity in a subject in need of such enhanced activity.

61. A pharmaceutical composition comprising the gene product encoded by  
the gene of Claim 55 for the purpose of enhancing telomerase activity in a subject in  
need of such enhanced activity.

5 62. A pharmaceutical composition comprising the gene of Claim 56, or a  
portion or a transcription product thereof, for the purpose of suppressing telomerase  
activity in a subject in need of such suppressed activity.

10 63. A pharmaceutical composition comprising the gene product encoded by  
the gene of Claim 56 for the purpose of suppressing telomerase activity in a subject in  
need of such suppressed activity.

15 64. A pharmaceutical composition comprising the protein of Claim 58 for  
the purpose of enhancing telomerase activity in a subject in need of such enhanced  
activity.

65. A gene encoding the protein of Claim 58.

20 66. A pharmaceutical composition comprising the gene of Claim 65 for the  
purpose of enhancing telomerase activity in a subject in need of such enhanced  
activity.

67. A pharmaceutical composition comprising the compound of Claim 59  
for the purpose of inhibiting telomerase activity in a patient in need of such decreased  
activity.

5        68. A method for activating endogenous telomerase for the purpose of  
extending the life span of a primary cell.

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